220 Laboratory Evaluation of Infectious Disease Emergencies

STEPHEN G. BAUM

The diagnostic work-up of any serious infection constitutes an emergency. The most innocuous-appearing respiratory infection can proceed to fatal meningitis in a matter of hours, and minor urinary tract symptoms can herald a syndrome of overwhelming sepsis. Recent publications have emphasized the development of new laboratory culture techniques for the rapid identification of infectious agents. At best, these techniques require several hours and are susceptible to many artifacts of specimen collection and storage prior to the time the sample reaches the laboratory.

Accurate assessment of the probable causes of serious infection can be made by the examining clinician within minutes to an hour after first seeing a febrile patient. This is accomplished by coupling carefully selected history and physical features with the proper collection of specimens and the use of a few simple staining techniques. The goal of this rapid diagnostic work-up is elucidation of the causative organism, allowing use of the *fewest* possible antibiotics that combine the proper antimicrobial spectrum with the least toxicity to the patient.

The choice of the right therapeutic agent is extremely important for several reasons. First, infections constitute the only medical conditions that may be susceptible to true cure rather than amelioration. Second, bacteria may develop resistance to an antibiotic with a frequency roughly proportional to the degree to which the antibiotic is used. Therefore, injudicious use of broad-spectrum antibiotics may shorten the time during which they remain useful by hastening the evolution of a new population of resistant microorganisms. Third, use of broad-spectrum antimicrobial therapy perturbs the normal flora, promoting overgrowth of opportunistic organisms such as fungi. The physician treating a patient with an infectious disease therefore has the capacity to alter the pathogenesis of diseases not only in the patient but also in the hospital population and the community at large. The emergent nature of infections, the possibility of bringing about real cure, and the potential ill effects of antibiotic misuse make it incumbent upon the physician to ascertain the etiology of the infection rapidly and accurately, and to use the *narrowest* spectrum of effective drugs.

The prime prerequisite for accurate laboratory work-up of any infectious disease is proper collection of appropriate specimens. Infectious disease consultants are frequently asked to review "sputum" specimens used in the initial diagnosis of a patient who has not responded well to therapy, only to find saliva rather than sputum. Whether used for staining and microscopic examination or for culture, specimens must be collected properly. Following is a discussion of the laboratory work-up of four commonly analyzed specimens: cerebrospinal fluid, sputum, urine, and blood. Many of the techniques described for collection and processing of these samples are also valid for specimens such as synovial and pleural fluid.

Analysis of Cerebrospinal Fluid in Central Nervous System Infection

Definition

The physician must have a low threshold for the performance of a lumbar puncture (LP) in cases of suspected meningitis. In children, the presence of fever, stiff neck, headache, and irritability are obvious justifications for an LP, while in the aged patient a stiff neck is frequently not found and fever accompanied by confusion or clouded sensorium may be the only signs of meningitis. If there is clinical evidence for increased intracranial pressure, a CT scan of the head should be performed before the LP.

Technique

The patient should be placed horizontally on a firm surface with the neck, legs, and back flexed in fetal position. The back should then be surgically prepared first with 70% alcohol and then with iodine. Start at the point the needle will enter and swab in a spiral motion, extending laterally to the flanks, cephalad to the midback, and caudad to the coccyx. This should be repeated twice, using fresh swabs saturated with iodine solution each time. Skin irritation from iodine may be reduced by thoroughly washing the back with alcohol at the end of the procedure.

After donning gloves, local anesthetic is introduced into the interspace between the second and third lumbar vertebrae. The exact positioning of the LP needle and method of its introduction are described in neurology or neurosurgery textbooks.

Several tubes should be available for the collection of cerebrospinal fluid (CSF). These should include a tube collected first for culture (0.5 to 1 ml) and others for chemical assay of protein and sugar (1 to 2 ml), serologic test for syphilis (1 ml), and, most important, a tube for gross inspection, microscopic cell count, differential cell count, and gram stain (1 to 2 ml). An additional tube of CSF should be saved for future assays.

Clinical Significance

In the diagnosis of meningitis there is no substitute for the gross and microscopic examination of cerebrospinal fluid.

Table 220.1
Diagnostic Significance of Cells in the Spinal Fluid

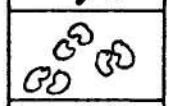
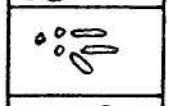
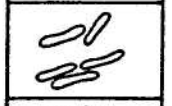
Cell type	Number (% total count)	Diagnostic interpretations
Erythrocytes	Changing number from early to later tube	"Traumatic tap"
	Constant: 5–200/mm³	Herpes simplex encephalitis
	Constant: 100–>1000/mm³	Mycotic aneurysm (subarachnoid bleed)
Leukocytes		
Mononuclear	5–500 (90–100%)[a]	Viral meningitis (mumps, enterovirus)
		Viral encephalitis (herpes simplex, varicella-zoster)
		Tuberculous meningitis
		Fungal (cryptococcal) meningitis
	>1000 (90–100%)	Lymphocytic choriomeningitis
	100–2000 (~50%)	Partially treated bacterial meningitis
		Brain abscess
Polymorphonuclear	5–2000 (>80%)	Bacterial meningitis (expect to see organisms on gram stain)
	100–2000 (~50%)	Partially treated bacterial meningitis

[a]Very early, polymorphonuclear leukocytes may outnumber mononuclear cells.

The color and clarity of the spinal fluid on *gross examination* should be reported. Usually it will either be "water clear," "yellow clear," "turbid," or "grossly bloody." A yellow color denotes either increased protein content or the presence of old, hemolyzed blood. Turbidity is due to the presence of cellular elements or numerous microorganisms. CSF that is clear to the naked eye may still contain cells and microorganisms, and therefore should always be examined microscopically.

On *microscopic examination,* white blood cells (WBC) should be enumerated in a standard WBC counting chamber using CSF that is fresh and *has not been centrifuged.* If cells are seen, no attempt should be made to differentiate cell types in the counting chamber. Even with the addition of a dye such as methylene blue, this method of differential counting is fraught with error. The fluid should then be centrifuged and the differential count done as soon as possible on the pellet, if visible, or on the bottom-most drop of CSF if there is no visible pellet. The drop of sedimented fluid is placed on a clean slide. It is not smeared, but is allowed to air dry. Two such preparations are made; one is heat fixed for gram stain and the other Wright stained for the differential count.

Table 220.2
Microorganisms in the Cerebrospinal Fluid

Stain findings	Morphology	Organism	Prototypic patient[a]
Gram-positive diplococci		Pneumococci	Normal adult
Gram-positive cocci in chains		Streptococci (group B)	Neonate
Gram-positive rods		Listeria	Immunosuppressed, postpartum patient
Gram-negative diplococci		Meningococci	Child 4–18 years old
Gram-negative coccobacilli		Hemophilus	Child 6 mos–4 years old
Gram-negative rods		Enterobacteriacea	Neonate, geriatric, neurosurgery patient
"Figure-eight" on India ink		Cryptococci	Normal, immunosuppressed adult

[a]Although there is a predisposition for a given type of organism to cause meningitis in certain patient populations, it should be remembered that any organism may at times cause disease in any type of patient.

An accurate differential can often be obtained from the gram-stained material. Interpretations of the WBC count and differential are given in Table 220.1. These are rough indices, and there is much overlap in counts and differentials between diseases.

Red blood cells (RBC) may be present in the CSF as a result of infectious or noninfectious processes involving the brain or subarachnoid space, or may be introduced into the CSF as a result of the lumbar puncture. The latter occurrence is called a "traumatic tap." This term does not imply that any difficulty was encountered in performing the spinal tap, but rather that a capillary was entered in the process, thereby carrying blood into the CSF. That this event has occurred is evidenced by a change in the amount of blood (either an increase or decrease) when a tube collected at the start of the LP is compared with one collected later in the procedure. If blood is apparent to the naked eye, this comparison can be made grossly. If blood is visible only under the microscope, counts must be done on an early and later tube. A constant amount of blood in all tubes indicates that the bleeding occurred before the LP. Infectious implications of blood in the CSF are given in Table 220.1.

Microorganisms may be detected by gram stain or India ink preparations. The etiology of over 80% of cases of bacterial meningitis can be determined by examining a gram-stained specimen of CSF. This procedure can be performed in 2 minutes and should never be left to a laboratory technician to be done later. The findings from the rapid method of gram staining are shown in Table 220.2 together with description and interpretation of some of the more common organisms that cause meningitis. For the India ink test, to one drop of sediment from centrifuged CSF on a slide, add one drop of ink. Cover the wet preparation with a coverslip and examine under "high dry" magnification. The finding of budding organisms, visible as bright spots in a "figure-eight" configuration against the dark background of india ink, indicates the presence of cryptococci (See Table 220.2). In the absence of budding, other visible elements should not be called cryptococci.

CSF for culture should be taken to the bacteriology laboratory as soon as possible. Pending culture, CSF can be stored in an incubator to maximize the viability of any organisms present.

Analysis of Sputum in Respiratory Tract Infection

Definition

Gram stain of the sputum is perhaps the most accurate way of diagnosing pulmonary infections. Because there are always organisms in the nasopharynx, culture of the sputum will often appear positive for some bacterial microorganism. This culture positivity may be misleading in cases of influenza, mycoplasma, or legionella pneumonia. In contrast, when anaerobes are the etiologic agents, as in aspiration pneumonia, their presence will not be revealed by routine sputum culture techniques. Examination of gram-stained sputum will demonstrate whether a bacterium is present as a predominant species and whether the sputum contains polymorphonuclear leukocytes suggestive of a bacterial infection. The presence of numerous large squamous cells, even when these are filled with bacteria, indicates that the specimen has been contaminated with saliva and is therefore inappropriate for diagnostic purposes.

Technique

Sputum can be collected either as a freshly coughed-up specimen or via aspiration through a nasotracheal tube following rapid instillation of 5 to 10 ml of normal saline. Transtracheal aspiration is only rarely necessary to obtain sputum.

A sample of sputum is spread on a slide with an applicator stick or by compressing the specimen between two slides. After the specimen has dried in air, it is heat fixed and gram stained as described for CSF, with one exception. A sputum specimen will have thick and thin areas. If one attempts to decolorize the thick areas completely, the thin areas may be over-decolorized. Therefore, one should leave the thickest areas of the sputum blue.

Clinical Significance

When examined under the microscope, the best area in which to search for organisms is one where the nuclei of the leukocytes are pink. In cases of bacterial pneumonia, one should note many polymorphonuclear leukocytes and a marked preponderance of one morphologic type of organism; that is, in pneumococcal pneumonia, one should see only gram-positive diplococci to the exclusion of almost all other bacterial forms. An exception occurs in cases of aspiration pneumonia, in which one sees many different types of organisms indicative of the many types that have been aspirated from the mouth. The implications of different patterns seen on sputum gram stain are given in Table 220.3.

Table 220.3
Diagnostic Significance of Findings on Sputum Gram Stain

Stain findings	Interpretation
Gram-positive diplococci & PMNs	Pneumococcal pneumonia
Gram-positive cocci in clusters & PMNs	Staphylococcal pneumonia
Gram-negative coccobacilli & PMNs	*Hemophilus influenzae* pneumonia
Gram-negative fat rods with bipolar staining & PMNs	Klebsiella pneumonia
Gram-negative slender rods & PMNs	Enterobacteriacea, pseudomonas pneumonia
Gram-negative diplococci & PMNs	*Branhamella catarrhalis* pneumonia (rarely, meningococcal)
Mixed gram-positive and -negative cocci and rods & PMNs	Aspiration pneumonia
Few bacteria seen; many PMNs or mononuclear cells	Influenza, mycoplasma, or legionella pneumonia
Mixed organisms with squamous cells	Specimen contaminated with saliva

PMNs = polymorphonuclear leukocytes.

Analysis of Urine in Urinary Tract Infection

Definition

Examination of urine for the presence of infection involves the finding of WBCs and/or bacteria. Unlike CSF, the urine usually contains some WBCs and is easily contaminated with bacteria. Hence, proper assessment requires special steps in the collection, preparation, and storage of the specimen. Using a few simple techniques, one can show that a urinary tract infection exists and make an educated guess at the probable offending organism, and therefore at the appropriate therapy. By repeating these procedures 24 hours after the start of treatment, one may also decide if the chosen therapy has been effective. In many cases, all of this can be accomplished before the microbiology laboratory has reported the results of the initial culture.

Technique

Patients from whom urine is to be examined for the presence of infection fall into two groups: those with and without indwelling urinary catheters. In patients without catheters, a midstream clean-voided specimen is recommended. The periurethral area should first be cleansed with a soap solution to get rid of contaminating resident flora, then rinsed with sterile water or saline to avoid the antibacterial effects of the soap when the urine is cultured. To avoid contamination of the urine by feces in female patients, it is essential that cleansing of the periurethral area be done from the urethral meatus toward the perineum and never in the reverse direction. In the male, it is important to retract the foreskin, if present, before cleaning the periurethral area. Cleansing agents other than soap, such as those containing hexachlorophene (e.g., pHi-so-Hex) and quaternary ammonium compounds (e.g., Zephiran) should be avoided. The former may dry out the urethra, making it susceptible to infection, and the latter are easily contaminated with pseudomonas. The importance of a midstream specimen is that the initial voiding washes out bacteria that normally reside in the distal urethra. In patients who cannot cooperate, urine may have to be obtained by passing a temporary catheter. This should be done using sterile technique and the cleansing procedures detailed above.

In the patient with an indwelling catheter, the proper method of obtaining urine is to swab the port with alcohol and aspirate urine using a sterile syringe. If no port is provided, a syringe and small-gauge needle can be used. In this case the distal *rubber* part of the closed drainage system should be cleaned with an alcohol pledget, and the rubber tubing should then be punctured and urine withdrawn. Make sure that the lumen of the tube draining the bladder is entered rather than the tube lumen that leads to the inflated balloon. To ensure this, the catheter must be punctured distal to the bifurcation or trifurcation. The tubing system should never be disconnected to obtain urine, as the "closed" modern urine collection systems play a major role in preventing infection in catheterized patients. The plastic part of the draining system should not be punctured because it will not self-seal. Urine for diagnosis of infection should never be obtained from the collection bag. This urine is not fresh and is often contaminated with bacteria, regardless of the presence or absence of urinary tract infection.

The urine *should not* be centrifuged before the gram stain. The gram stain is prepared as follows. A drop of unspun urine is placed on a clean slide and allowed to dry without smearing. The specimen is then heat fixed and stained as described for CSF (Figure 220.1). An approximation of WBCs is usually made by centrifuging the urine and examining a drop of sediment under the high dry objective of the microscope without prior staining.

After staining, urine should be sent for culture to confirm the presence, type, and number of bacteria. If the urine is not cultured immediately, it must be stored in the refrigerator so that high colony counts do not merely reflect bacterial replication after the urine was collected.

Clinical Significance

A colony count of more than 100,000 (10^5) organisms per milliliter correlates well with other evidence of infection. It is unusual to see bacteria on gram stain of the urine unless the organisms number more than 100,000/ml. Putting these two facts together, if one sees bacteria in gram-stained urine, one can assume that the organisms exceed 100,000/ml and therefore the likelihood of infection is high.

The two most common bacterial types are gram-negative rods or gram-positive cocci. The latter may be seen in short chains, signifying that they are streptococci, most probably enterococci. Gram-positive cocci in clusters are probably staphylococci, and *S. saprophiticus* is now recognized as a common lower urinary tract pathogen. Gram-negative rods are usually not further identifiable by gram stain except by experts. Gram-negative cocci may belong to the genus *Acinetobacter.*

Exact enumeration of WBCs in the urine is not very helpful in establishing the presence of urinary tract infection. From 1 to 10 WBCs per high power field may be found in noninfected urine; between 10 and 50 is suggestive of infection. Some studies have indicated that, as with bacterial enumeration, the presence of any WBCs on unspun urine carries clinical significance.

The speciation of the organism may be useful in identifying its site of origin and whether it is the result of relapse

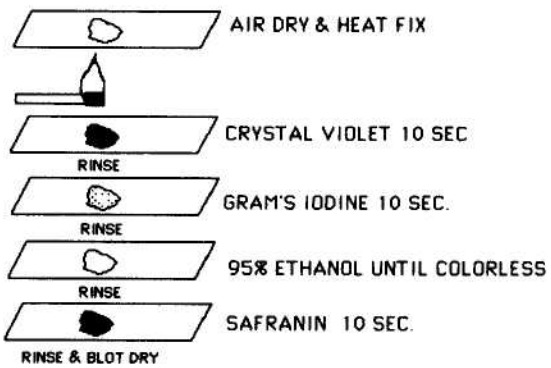


Figure 220.1
Gram-stain technique.

or reinfection. Further laboratory work-up will also provide information about the antibiotic sensitivity of the organism. However, one should not necessarily alter the initial therapy if the patient is doing well and if a repeat urine gram stain fails to reveal organisms. Many antibiotics are concentrated in the urine and are effective even when in vitro sensitivity tests would predict failure.

The estimation by gram stain of $>10^5$ organisms per milliliter of urine yields a high degree of certainty that the patient has a urinary tract infection. Conversely, not finding this many organisms cannot be used as proof that a patient is free of infection. The two groups in whom relatively low bacterial counts occur in the presence of infection are females with lower tract infections (the so-called urethral syndrome) and patients with indwelling urinary catheters. In catheterized patients the replication time for the organism is decreased because bladder urine exits immediately via the catheter. In females with lower urinary tract infections, in whom *S. saprophiticus* is a common pathogen, frequent voiding may similarly be responsible for relatively lower colony counts. The physician must use clinical criteria, the presence of pyuria, and sometimes the recurrent finding of low numbers of the same organism to decide if the patient is infected.

Analysis of Blood in Bacteremia

Definition

During the course of many infections, microorganisms enter the bloodstream. In some infected patients, the blood may be the only site from which organisms can be cultured. Blood cultures should be taken on all hospitalized patients with severe infections of the urinary tract, lungs, abdominal viscera, bones, central nervous system, or vascular tree.

Technique

In infections other than endocarditis, two or three sets of blood cultures are sufficient. When endocarditis is suspected, five sets of cultures are recommended. Each set of blood cultures (usually two bottles per set: one for aerobic and one for anaerobic culture) should be from a different venipuncture. Blood cultures should be drawn over a period of several hours to a day. The practice of drawing one large syringe of blood and aliquoting it into many blood culture bottles is inadequate. If contamination occurs before the aliquoting, all blood cultures will be positive for the same organism but this will not have the same diagnostic significance as multiple positive cultures derived from several sites over different periods of time.

The arms are the preferred site for the drawing of blood cultures. Either the antecubital fossa or a more distal spot may be used. The area should be prepared first with 70% alcohol and then with an iodine-containing solution, and allowed to dry before the venipuncture is performed. If it is necessary to palpate the area to locate a vein, the venipuncturist should either wear sterile gloves or prepare his or her own fingers so as not to contaminate the area. Once the blood is drawn, it should be transferred to the appropriate bottles without changing needles or swabbing the tops of the bottles with disinfectant. The needle used in the venipuncture and the bottle tops should be sterile. Any additional handling increases the chances for contamination or for the introduction of bactericidal material into the culture bottle. Be sure to place an adequate volume of blood (5–10 ml) in each culture bottle.

Clinical Significance

Routine blood cultures will usually permit a diagnosis to be reached. Two additional techniques may prove rapidly useful in cases of suspected bacteremia. The first is a search for pustular lesions on the extremities. Pustular or acneiform lesions are common on the face and trunk of normal uninfected people. However, they are rare on the extremities, especially on the hands and feet, and may be a clue to bacteremic disease. If found, these lesions should be gently swabbed with alcohol, unroofed or aspirated with a syringe and needle, and the contents gram stained. If organisms are identified, an immediate tentative diagnosis can be made. Material can then be sent for confirmatory culture and determination of antibiotic sensitivity. This approach is useful in cases of gonococcal, meningococcal, and pneumococcal sepsis.

A second technique is a gram stain of the "buffy coat." When whole blood is centrifuged, as when determining the hematocrit, the WBCs are concentrated as a white layer at the interface of plasma and erythrocytes. This layer is called the buffy coat. In the presence of high-level bacteremia, circulating leukocytes may contain phagocytized organisms. One can break the microhematocrit tube at the area of the buffy coat, express a drop of WBC-rich blood onto a slide, smear it, and gram stain it. Although this is a low-yield procedure (i.e., it will be positive in about 5% of bacteremias, more so staphylococcal and meningococcal bacteremias where blood colony counts may be very high), it is easy to do and can give immediate diagnostic results.